

In the light of potential applications [1-4] of lanthanide complexes and objectives of the present work, La(III), Ce(III), Pr(III), Nd(III) and Sm(III) complexes with 2-formylpyridine benzoylhydrazone (FPBH) are synthesized and characterized based on physico-chemical techniques and spectral methods viz., IR, UV-Visible spectroscopy. Electrochemical properties of all complexes are studied by cyclic voltammetry. Binding interactions of metal complexes with calf-thymus DNA are carried out by using absorption spectroscopy. DNA cleavage activities of the complexes are investigated using gel electrophoresis experiments.

Synthesis and characterization of FPBH (**Fig 4.1**) are given in Chapter 2. The ligand is synthesized by using corresponding precursors.

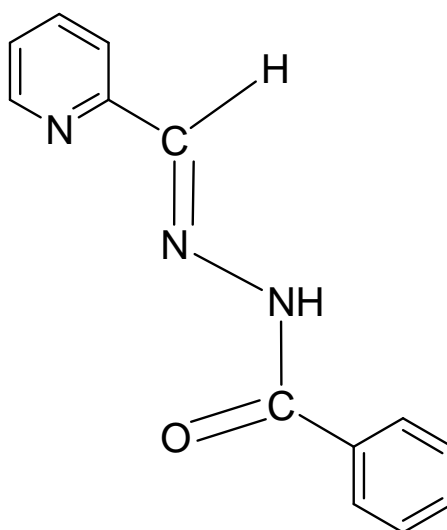


Fig 4.1: The structure of FPBH

a. Physico-chemical properties

Syntheses of lanthanide(III) complexes are given in Chapter-2 (Section 2 iii). All the complexes are stable at room temperature, non-hygroscopic, less soluble in water, methanol, ethanol and readily soluble in DMF and DMSO. The analytical data are consistent with the proposed molecular formulae of complexes. Physical properties viz.. colour of the complex, melting points and percentage of yield are given in **Table**

4.1.

b. Conductivity measurements

All the complexes are freely soluble in dimethylformamide (DMF), hence the solutions of these metal complexes were prepared in DMF to perform conductivity measurements. Milli-molar solutions (10^{-3}M) were prepared in 25-ml standard flasks by dissolving appropriate quantity of the metal complex in DMF and then the solutions were transferred into clean dry 100-ml beakers. Conductivity values of the solutions were measured at room temperature. The molar conductivity values (12-19 $\Omega^{-1}\text{cm}^2\text{mol}^{-1}$) of the complexes suggest that these are non-electrolytes [5].

Table 4.1 Colors, analytical data, molar conductivities and melting points of the FPBH ligand and its Lanthanide(III) complexes.

Compound	Colour	M.P °C	Yield %	Found (cal %)			ΔM^*
				C	H	N	
FPBH	White	103-105	75	69.42(69.32)	4.86(4.92)	18.42(18.66)	...
[La(FPBH) ₂ (NO ₃) ₃]	White	230-232	69	40.16 (40.27)	2.80 (2.86)	16.14 (16.26)	16.16
[Ce(FPBH) ₂ (NO ₃) ₃]	Yellow	210-212	74	40.35(40.21)	2.76 (2.86)	16.31 (16.23)	12.54
[Pr(FPBH) ₂ (NO ₃) ₃]	Light Green	222-224	71	40.05(40.16)	2.90 (2.85)	19.26 (16.21)	14.59
[Nd(FPBH) ₂ (NO ₃) ₃]	Light Pink	232-234	67	39.85 (40.00)	2.91 (2.84)	16.37 (16.15)	19.86
[Sm(FPBH) ₂ (NO ₃) ₃]	Light white	217-219	65	39.82 (39.69)	2.76 (2.82)	15.97 (16.02)	11.84

*($\Omega^{-1} \text{ cm}^2 \text{ mol}^{-1}$)

c. Electronic Spectra

Lanthanide complexes exhibit various colours due to the redistribution of electrons in the partially filled f-orbitals and referred as f-f transitions. In absence of the ligands around the metal, the energy, of all seven f-orbitals of a lanthanide metal ion are equal and are degenerate. The presence of ligands will result in splitting in the energy levels of these orbitals. In metal complexes some orbitals will interact more strongly than others. The exact form of interaction and energies of f-orbitals depend on the arrangements of ligands around the metal ions. In UV-visible, near IR region of electromagnetic radiation, the transition associated with electronic energy levels of the compound under investigation can be identified. The electronic spectral data of lanthanide complexes are recorded in dimethylformamide (DMF).

Electronic spectrum of the free ligand (FPBH) in UV region shows an intense band at 298 nm and weaker band at 371 nm which are assigned to the $\pi \rightarrow \pi^*$ and $n \rightarrow \pi^*$ transition respectively. These are slightly shifted to higher or lower energy levels in absorption spectra of the lanthanide complexes. In visible region, Pr(III), Nd(III), Sm(III) complexes show several important f-f spectral bands (Fig. 4.2). Generally, the lanthanide complexes exhibit red shift of all the f-f spectral bands (Figure 4.3) relative to the corresponding Ln(III) aquo ion. According to Jorgenson the shifts have been attributed to the effect of crystal field upon interelectronic repulsion(B) between the 4f electrons and is related to covalence in the metal-ligand bond. The values of the bonding parameters are shown in Table 4.2. The positive and negative values of δ and $b^{1/2}$ for a complex correspond to covalent and ionic characters, respectively. A nephelauxetic ratio (β) of less than unity and positive

values of Sinha's parameter (δ) and the bonding parameter ($b^{1/2}$) suggest the occurrence of some covalent character in the metal– ligand bond [6, 7]. the small ($\delta\%$) values of the complexes indicate weak covalent bonding in the complexes. the small $b^{1/2}$ values suggest week participation of 4f orbitals in bonding.

Nephelauxetic ratio (β) :

$$\beta = \frac{\nu_c}{\nu_f}$$

(where ν_c & ν_f = wave numbers of f-f transition in spectra of metal complex and free metal ion in solvent)

Bonding Parameter ($b^{1/2}$) :

$$b^{1/2} = \left[\frac{1}{2}(1-\beta) \right]^{1/2}$$

Sinha's Covalency parameter ($\delta\%$)

$$\delta \% = \frac{1-\beta}{\beta} \times 100$$

Covalency angular overlap parameter (η):

$$\eta = \frac{1-\beta^{1/2}}{\beta^{1/2}}$$

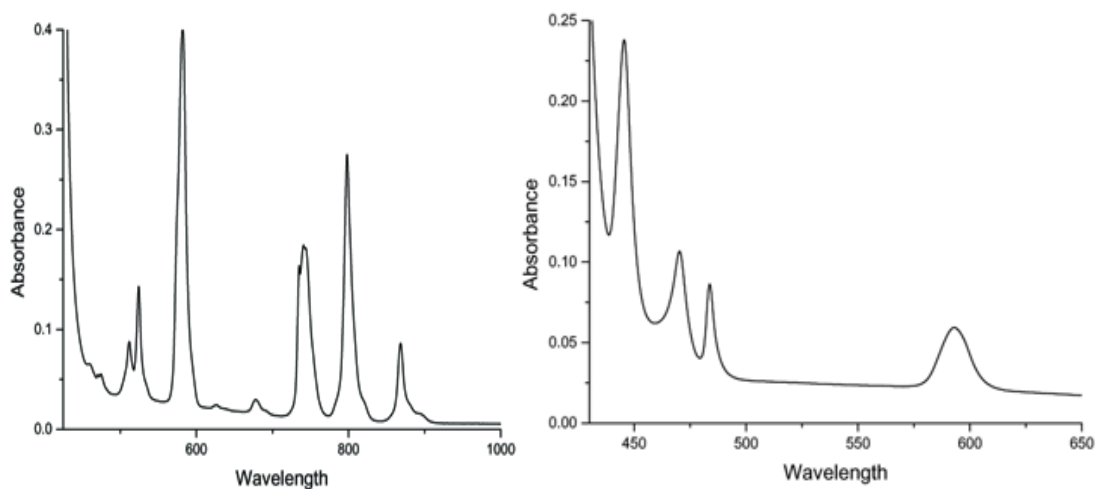


Fig 4.2: Electronic spectra of (A) Nd(III) complex, (B) Pr(III) complex

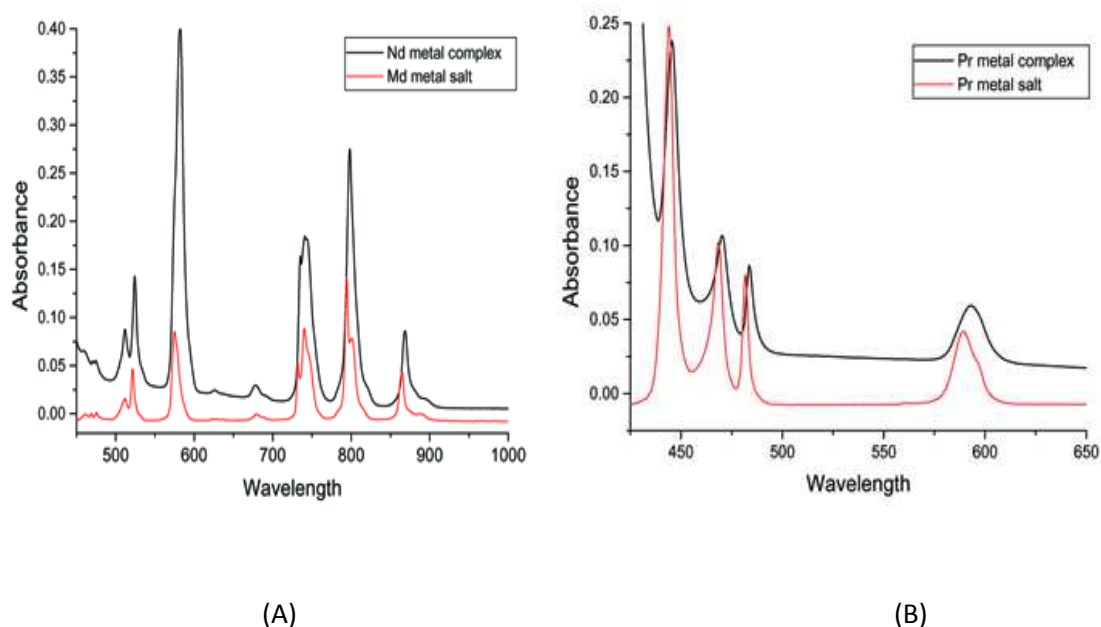


Fig 4.3:Electronic absorption spectra of (A) $\text{Nd}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (red) & $[\text{Nd}(\text{FPBH})_2(\text{NO}_3)_3]$ (black) and (B) $\text{Pr}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ (red) & $[\text{Pr}(\text{FPBH})_2(\text{NO}_3)_3]$ (black) in the visible region

Table 4.2 Electronic spectral data of the lanthanide complexes and related bonding parameter

complexes	Frequency (cm ⁻¹)		Red shift (cm ⁻¹)	Assignment	Covalent Parameter
	Lanthanide Aqua ion	Lanthanide complexes			
[Pr(FPBH) ₂ (NO ₃) ₃]	16,977	16,867	110	³ H ₄ → ¹ D ₂	β =0.9958
	20,746	20,659	87	³ H ₄ → ³ P ₀	δ% =1.26
	21,367	21,275	92	³ H ₄ → ³ P ₁	b ^{1/2} =0.046
	22,522	22,474	48	³ H ₄ → ³ P ₂	η =0.0021
[Nd(FPBH) ₂ (NO ₃) ₃]	11,560	11,518	42	⁴ I _{9/2} → ⁴ F _{3/2}	β =0.9957
	12,594	12,534	60	⁴ I _{9/2} → ⁴ F _{5/2}	δ % =0.43
	13,513	13,498	15	⁴ I _{9/2} → ⁴ F _{7/2} , ³ S _{3/2}	b ^{1/2} =0.046
	17,391	17,186	205	⁴ I _{9/2} → ⁴ G _{5/2} , ² G _{7/2}	η =0.0022
	19,120	19,083	37	⁴ I _{9/2} → ⁴ G _{7/2}	
	19,569	19,531	38	⁴ I _{9/2} → ⁴ G _{9/2}	
[Sm(FPBH) ₂ (NO ₃) ₃]	24,038	23,985	53	⁶ H _{5/2} → ⁶ P _{5/2}	β =0.9989
	22,675	22,647	28	⁶ H _{5/2} → ⁴ G _{9/2}	δ% =0.11
	21,598	21,596	2	⁶ H _{5/2} → ⁴ I _{13/2}	b ^{1/2} = 0.023
	20,876	20,873	3	⁶ H _{5/2} → ⁴ I _{11/2}	η = 0.0006
	20,040	20,000	40	⁶ H _{5/2} → ⁶ G _{7/2}	

d. IR Spectroscopy

The FT- IR spectra of complexes in the region $4000-400\text{ cm}^{-1}$ are analysed in comparison with the spectrum of metal free FPBH. The characteristic IR peaks of FPBH and its lanthanide complexes are given in Table 4.3. IR spectra of the five complexes are strikingly similar in relative positions and intensities of the peaks, which suggest a close structural relationship among the compounds. The IR spectrum of the free ligand shows strong band at 1663 cm^{-1} , which is attributable to stretching vibration of the carbonyl group ($\nu(\text{C}=\text{O})$). IR Spectra of cerium complex is given in Fig 4.4

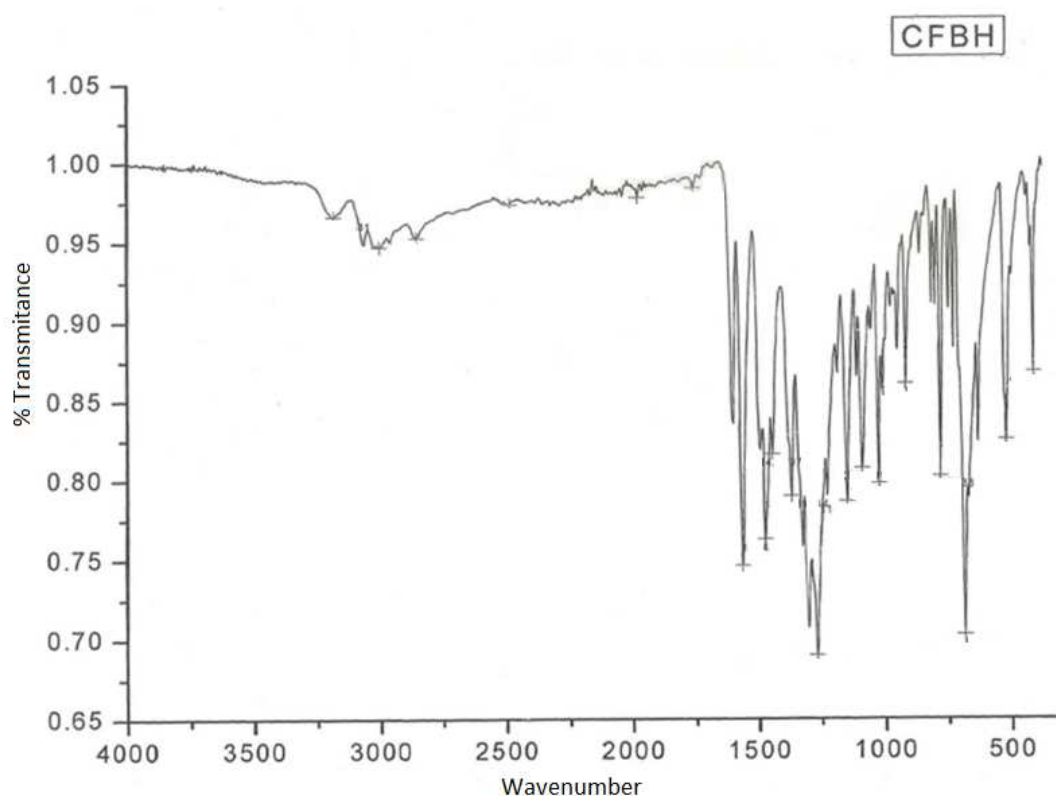


Fig 4.4: IR spectrum of $[\text{Ce}(\text{FPBH})_2(\text{NO}_3)_3]$ complex in KBr disc

The vibrational band at 1582 cm^{-1} can be assigned to the $\nu(\text{C}=\text{N})$ of azomethine. In the IR spectra of their lanthanide (III) complexes, the $\nu(\text{C}=\text{O})$ and $\nu(\text{C}=\text{N})$ shift by 31–39, 12–19. The shifts of the $\nu(\text{C}=\text{O})$ and $\nu(\text{C}=\text{N})$ vibrations of the bands towards lower wave numbers in spectra of complexes indicate participation of the carbonyl oxygen and azomethine nitrogen in coordination to the metal ion [8]. The vibrational band at 3184 cm^{-1} can be assigned to the $\nu(\text{N-H})$ for the free ligand. The $\nu(\text{N-H})$ band is observed in the range $3187\text{--}3235\text{ cm}^{-1}$ for the complexes. Observance of the $\nu(\text{C}=\text{O})$ and $\nu(\text{N-H})$ bands in the IR spectra of complexes indicates that the FPBH acts as neutral to tridentate ligand in the complex formation. The pyridine ring in-plane deformation mode is observed at 624 cm^{-1} in the spectrum of ligand. These bands are shifted to higher wave numbers ($630\text{--}632\text{ cm}^{-1}$) in the spectra of lanthanide complexes indicating coordination of the heterocyclic aromatic nitrogen [8].

The absorption bands assigned to the coordinated nitrate groups (C_{2v}) are observed at about $1474\text{--}1476\text{ cm}^{-1}$ (ν_1), $1295\text{--}1307\text{ cm}^{-1}$ (ν_4), $1024\text{--}1026\text{ cm}^{-1}$ (ν_2) and 817 cm^{-1} (ν_3) [14] for the nitrate complexes. The frequency separation [$\Delta\nu = \nu_1 - \nu_4$] between the asymmetric and symmetric stretching of this group can be made to distinction between these binding states. While the separation of the two bands is $169\text{--}179\text{ cm}^{-1}$, suggest that NO_3^- acts as bidentate ligand [9, 10]. No peak is present at 1384 cm^{-1} in IR spectra of complexes indicating the absence of ionic nitrate (D_{3h} symmetry), which is in agreement with the results of the conductivity experiments. The new bands observed in $416\text{--}423$ and $541\text{--}546\text{ cm}^{-1}$ regions in the spectra of complexes are assigned to $\nu(\text{Ln-O})$ and $\nu(\text{Ln-N})$ vibrations respectively.

Table 4.3 Infrared spectral data (cm⁻¹) for the FPBH ligand and its lanthanide(III) complexes

Compound	ν(N-H)	ν(C=O)	ν(C=N)	ν(NO ₃ ⁻)				
				ν ₁ (NO ₃ ⁻)	ν ₄ (NO ₃ ⁻)	ν ₂ (NO ₃ ⁻)	ν ₃ (NO ₃ ⁻)	ν ₁ -ν ₄
FPBH	3184	1663	1582
[La(FPBH) ₂ (NO ₃) ₃]	3235	1632	1570	1476	1297	1026	817	179
[Ce(FPBH) ₂ (NO ₃) ₃]	3183	1628	1563	1474	1295	1024	816	179
[Pr(FPBH) ₂ (NO ₃) ₃]	3202	1624	1565	1476	1307	1024	818	169
[Nd(FPBH) ₂ (NO ₃) ₃]	3188	1626	1564	1474	1296	1024	817	178
[Sm(FPBH) ₂ (NO ₃) ₃]	3201	1624	1563	1474	1296	1025	817	178

e. Thermo gravimetric (TG) analysis

Thermogravimetric (TG) and differential thermal analyses(DTA) of lanthanide complexes of FPBH are carried out within the temperature range from ambient temperature up to 800⁰ C under nitrogen (inert) gas flow with heating rate 10°C/min.

All the complexes showed similar thermal decomposition. The TGA curve of the complex undergoes two-stage changes. All Ln(III) complexes are thermally stable up to 204⁰C indicating absence of lattice or coordinated water and solvent molecules. The first stage of decomposition in the range 205-303⁰ C is due to loss of three nitrate ions with a weight loss of 24.06% is consistent with theoretical value (24.00%). The second stage of decomposition in the range 304- 800 corresponds to the elimination and/or decomposition of free ligand [11].

g. Electrochemical studies

Redox behaviour of the complexes has been investigated by cyclic voltammetry using 0.1M tetrabutylammonium hexafluorophosphate (TBAHEP) as supporting electrolyte. The cyclic voltammetric profiles of [Ce(FPBH)₂(NO₃)₃] complex are given in Fig. 4.5. A plot of i_p vs $v^{1/2}$ (scan rate) is linear (Fig. 4.6) pointing towards diffusion controlled nature of reduction wave. In the reverse scan there is no anodic peak confirming the irreversible nature of electrode process. All these facts pointed towards the diffusion-controlled nature of the electrode process.

The cyclic voltammograms of La(III), Ce(III), Pr(III), Nd(III) and Sm(III) gave irreversible waves. Their cathodic peak potentials are found to be -0.916

[La(III)], -0.859 [Ce(III)], -0.856[Pr(III)], -1.025[Nd(III)] and -0.962[(Sm(III)]. This is due to the reduction of Ln(III) to Ln(II) couple[12].

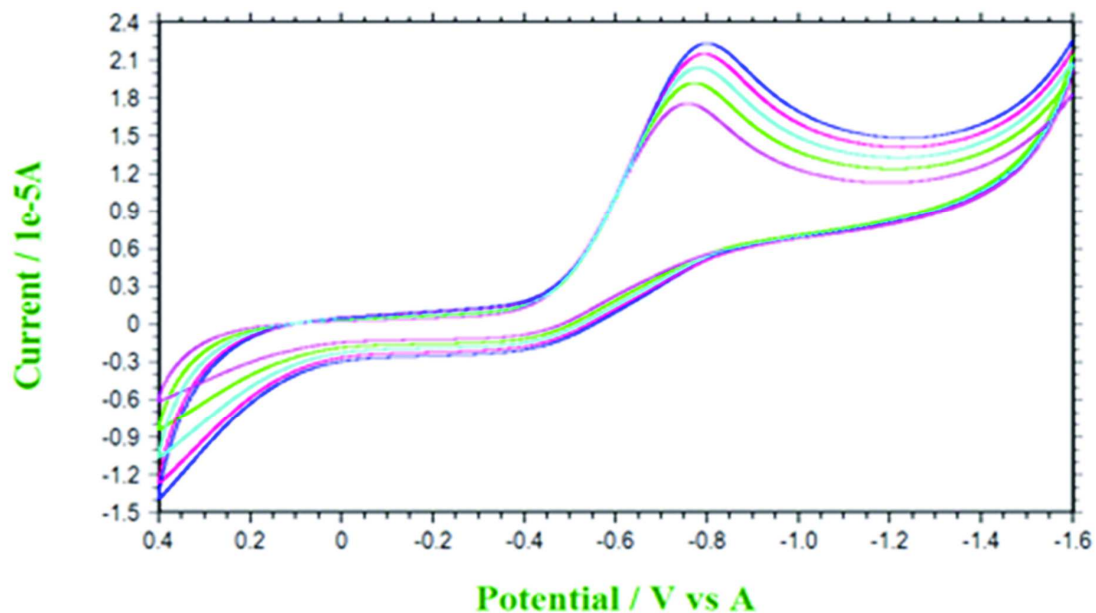


Fig. 4.5: Cyclic voltammetric profiles of the cerium complex at different scan rates 25-100 mV/s.

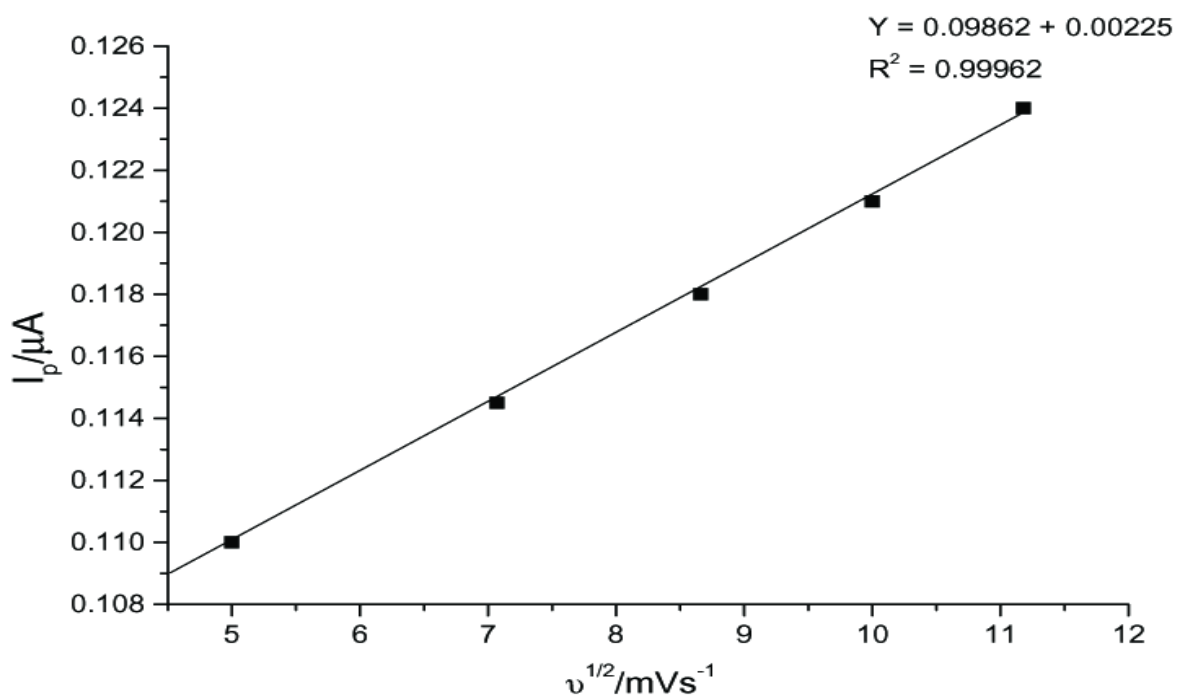


Fig 4.6: Plot of peak current vs scan rate for cerium complexes.

h. DNA binding studies

Electronic absorption spectroscopy is an effective method for examining the interaction of DNA with metal complexes. Hyperchromic and hypochromic effects are the spectral changes when a complex interacts with DNA and forms a new complex. In general, a complex binding with DNA through intercalation usually results in hypochromism and bathochromism of the absorption band due to the intercalative mode involving a strong π - stacking interaction between the aromatic chromophore and base pairs of DNA [13]. The binding interaction of complexes with CT-DNA was monitored by comparing their absorption spectra with and without CT-DNA. All the complexes exhibit an intense absorption band in 291-293 nm region attributed to $\pi \rightarrow \pi^*$ transition. Absorption spectra of $[\text{La}(\text{FPBH})_2(\text{NO}_3)_3]$ in the absence and in presence of CT-DNA are shown Fig. 4.7. The metal-free hydrazone less DNA binding activity compare to lanthanide-hydrazone complexes. The intrinsic binding constants (K_b), was determined by using the equation,

$$[\text{DNA}] / (\varepsilon_a - \varepsilon_f) = [\text{DNA}] / (\varepsilon_b - \varepsilon_f) + 1 / K_b(\varepsilon_b - \varepsilon_f) \quad \text{-----(1)}$$

Where $[\text{DNA}]$ is the concentration of DNA in base pairs, ε_a , ε_b and ε_f are apparent extinction coefficient ($A_{\text{obs}}/[\text{M}]$), the extinction coefficient for the metal (M) complex in the fully bound form and the extinction coefficient for free metal (M) respectively. A plot of $[\text{DNA}] / (\varepsilon_a - \varepsilon_f)$ versus $[\text{DNA}]$ gave a slope of $1/(\varepsilon_b - \varepsilon_f)$, and vertical intercept equal to $1 / K_b(\varepsilon_b - \varepsilon_f)$; K_b was calculated from these values. The binding constants (Table 4.4) for lanthanide complexes are almost similar and independent of metal ion. This observation suggests that the complexes do not bind DNA via coordination (No

direct Metal- DNA bond formation). On addition of DNA, the absorbance of the complexes decreases (hypochromism). Negligible shift (0.5-1.5 nm) in band position is observed for lanthanide complexes [14]. These observations suggest groove binding of complexes to DNA.

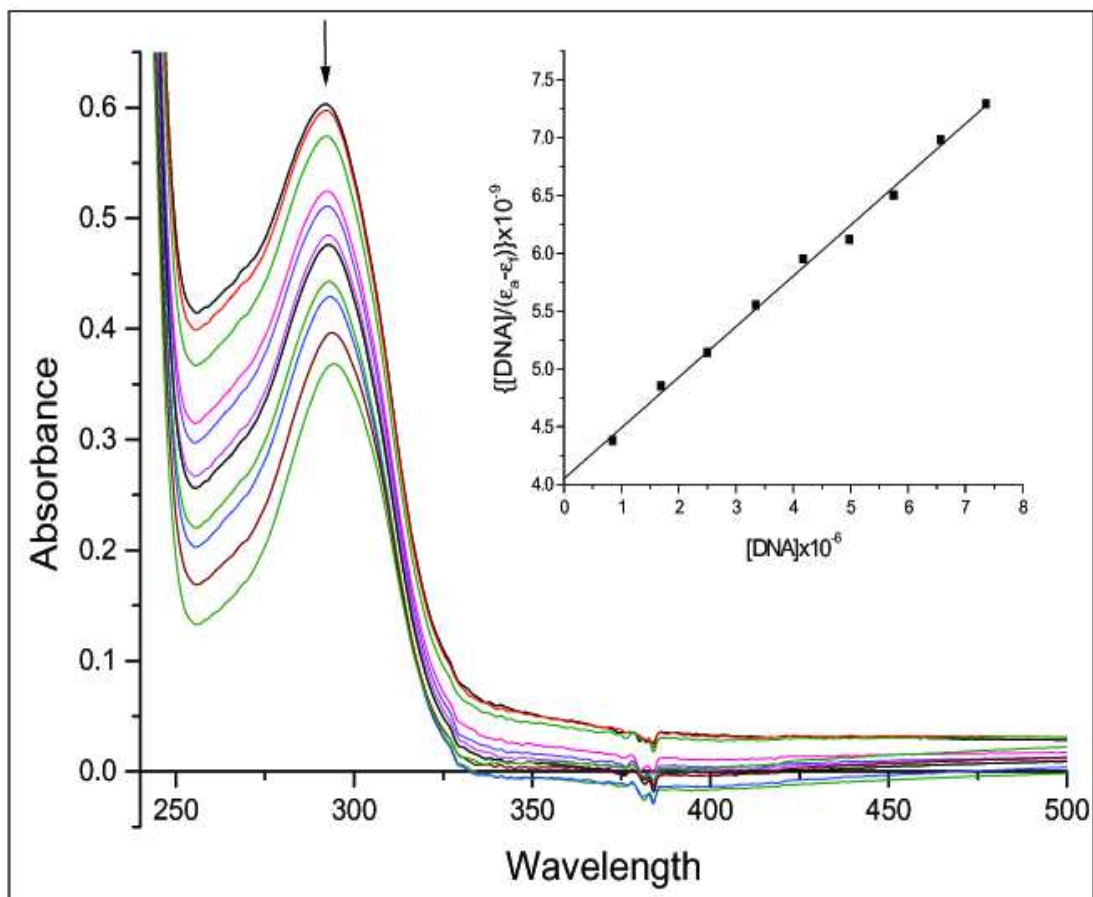


Fig 4.7: Absorption spectra of [La(FPBH)₂(NO₃)₃] in the absence and in the presence of increasing concentration of CT-DNA; top most spectrum is recorded in the absence of DNA and below spectra on addition of 10 μl DNA each time.

Table 4.4. Electronic absorption data upon addition of CT-DNA to the complexes

Compound	λ_{max} (nm)		$\Delta\lambda$	H(%)	K_b (M^{-1})
	Free	bound			
[La(FPBH) ₂ (NO ₃) ₃]	290	291	1	39.30	1.42x10 ⁵
[Ce(FPBH) ₂ (NO ₃) ₃]	291	292	1	32.50	1.40x10 ⁵
[Pr(FPBH) ₂ (NO ₃) ₃]	291	292	1	28.18	1.38x10 ⁵
[Nd(FPBH) ₂ (NO ₃) ₃]	292	293.5	1.5	41.54	1.53x10 ⁵
[Sm(FPBH) ₂ (NO ₃) ₃]	291	291.5	0.5	42.56	1.56x10 ⁵

i. DNA Cleavage studies

Nuclease activity of lanthanide complexes derived from 2-formylpyridine benzoylhydrazone(FPBH) has been studied by agarose gel electrophoresis using pBR 322 plasmid DNA in Tris-HCl/NaCl (50mM/5mM) buffer(pH-7) in the presence and in absence of H₂O₂ as an oxidant at micro molar concentration for 30 min incubation period at 37° C. In the presence of H₂O₂ the super coiled DNA (form I) is changed into nicked form (form II), which is further cleave in to linear form (form III). In the absence of oxidant, the complexes exhibit significant DNA cleavage activity (lanes 3 and 5 in Fig. 4.8 and lanes 3, 5 and 7 in Fig 4.9). In the presence of H₂O₂ the complexes cleave DNA more effectively [lanes 4 and 6 in Fig 4.9 lanes 4, 6 and 8 in Fig. 4.10], which due to the reaction of hydroxyl radical with DNA like Fenton mechanism [15]. These hydroxyl free radicals participate in the oxidation of the deoxyribose moiety [16]. The order of activity is as follows

Pr complex > Sm complex > Nd complex > Ce complex > La complex

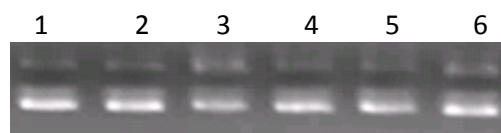


Fig 4.8: Agarose gel (0.8%) showing results of electrophoresis of 1 μ l of pBR322 Plasmid DNA; 4 μ l of Tris–HCl/NaCl (50 mM/5 mM) buffer (pH-7); 2 μ l of complex in DMF(1×10^{-3} M); 11 μ l of sterilized water; 2 μ l of H₂O₂ (total volume 20 μ l) were added, respectively, incubated at 37°C (30 min);

Lane 1: DNA control; Lane 2: DNA control + H₂O₂; Lane 3: Lanthanum complex+ DNA ; Lane 4: Lanthanum complex + DNA + H₂O₂; Lane 5: Cerium complex+ DNA; Lane 6: Cerium complex+ DNA + H₂O₂

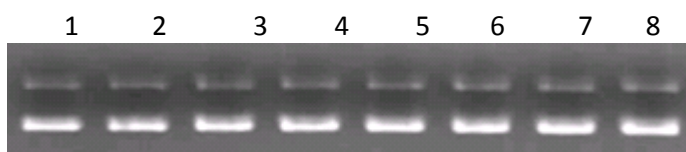


Fig 4.9: Agarose gel (0.8%) showing results of electrophoresis of 1 μ l of pBR322 Plasmid DNA; 4 μ l of Tris–HCl/NaCl (50 mM/5 mM) buffer (pH-7); 2 μ l of complex in DMF(1×10^{-3} M); 11 μ l of sterilized water; 2 μ l of H₂O₂ (total volume 20 μ l) were added, respectively, incubated at 37°C (30 min);

Lane 1: DNA control; Lane 2: DNA control + H₂O₂; Lane 3: Praseodymium complex+ DNA; Lane 4: Praseodymium complex+ DNA + H₂O₂; Lane 5: Neodymium complex+ DNA; Lane 6: Neodymium complex+ DNA+H₂O₂; Lane 7: Samarium complex+ DNA; Lane 8: Samarium complex + DNA+H₂O₂.

Conclusions

La(III), Ce(III), Pr(III), Nd(III) and Sm(III) complexes of 2-formylpyridine benzylhydrazone(FPBH) have been synthesized and characterized. Physico-chemical and spectral studies reveal that the complexes have general formula $[Ln (FPBH)_2 (NO_3)_3]$ (where Ln = La, Ce, Pr, Nd, and Sm). FPBH acts as neutral tridentate ligand and NO_3^- acts as bidentate ligand. Two FPBH ligands occupy six coordination sites and three NO_3^- ligands another six coordination sites to form 12- coordinate mono nuclear complexes.

References

1. E. Preshagen, E. Borbas, *Coord. Chem. Rev.* 273-274 (2014) 30.
2. C. M. G. Dos santos, J. H. Andrew, J. Q. Susan, T. Gunnlaugsson, *Coord. Chem. Rev.* 252 (2008) 2512.
3. B. Melanie, K. Lilan, J. L. Nicholas *Chem. Soc. Rev.* 35 (2006) 557.
4. C.A. Barta, S.B. Krishna, J. Jesica, K.H. Thomspn, M.W. Kishore, O. Chris *Dalton Trans.* (2007) 5019
5. W. J. Geary, *Coord. Chem. Rev.* 7 (1971) 81.
6. S. P. Sinha, *Spectrochim Acta* 22 (1966) 57.
7. K. Iftikar, M. Sayeed and N. Ahmad, *Bull. Chem. Soc. Japan* 55 (1982) 2258.
8. K. Nakamoto, *Infrared and Raman Spectra of Inorganic and Coordination Compounds*, fourth ed., Wiley, New York, 1986.
9. N. F. Curtis, Y. M. Curtis, *Inorg. Chem.* 4 (1965) 804.
10. P. Yan, W. Sun, G. Li, C. Nei, T. Gao, Z. Yue, *J. Coord. Chem.* 60 (2007) 1973.
11. X. M. Shi, R. R. Tang, G. L. Gu, K. L. Huang, *Spectrochim. Acta.* 72A (2009) 198.

12. Ch. Jagadeeswara Rao, K. A. Venkatesan, K. Nagarajan, T. G. Srinivasan, P.R. Vasudeva Rao, *J. Nucl. Mater.* 399 (2010) 81.
13. E .C. Long, J. K. Barton, *Acc. Chem. Soc. Res.* 23 (1990) 271.
14. S. Ramakrishnan, E Suresh, A. Riyasdeen, M. A. Akbarsha, M. Palaniandavar. *Dalton Trans.* 40 (2011) 3245.
15. Y. M. Song, J. P. Xu, L. Ding, Q. Hou, J. W. Liu, Z. L. Zhu, *J. Inorg. Biochem.* 103 (2005) 396.
16. W.K Pogozeleski, T.D Tullius, *Chem. Rev.* 98 (1998)1089.